Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis

Bloemberg, Guido V ; Keller, Peter M ; Stucki, David ; Trauner, Andrej ; Borrell, Sonia ; Latshang, Tsogyal ; Coscolla, Mireia ; Rothe, Thomas ; Hömke, Rico ; Ritter, Claudia ; Feldmann, Julia ; Schulthess, Bettina ; Gagneux, Sebastien ; Böttger, Erik C

Abstract: Treatment of multidrug-resistant Mycobacterium tuberculosis is a challenge. This letter describes the emergence of resistance to new therapies, bedaquiline and delamanid.

DOI: https://doi.org/10.1056/NEJMc1505196

a diagnosis of multiple primary tumors.[4] In our patient, we had identified two different truncating germline *NTHL1* mutations in trans: the above-mentioned c.268C→T,p.Q90* mutation and c.709+1G→A, which results in abnormal splicing (Fig. 1B, and Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). We used several methods to study the mutation profile of the tumors (Table S1 and Fig. S2 and S3 in the Supplementary Appendix). In so doing, we extended the previous report[4] to include benign and malignant extracolonic tumors. The mutation spectrum was significantly shifted in favor of C:G→T:A transitions (Fig. S2 in the Supplementary Appendix), as reported previously.[4] Among other driver mutations, we identified the frequently reported[5] *FGFR3* mutation c.742C→T,p.R248C in one seborrheic keratosis (Fig. S3 in the Supplementary Appendix).

A single *NTHL1* mutation, p.Q90*, was identified by investigators in Nijmegen, the Netherlands,[4] which is only 150 km from Dortmund, Germany, where the paternal relatives of our patient were born. This finding suggests that the p.Q90* allele was inherited paternally. The c.709+1G→A mutation has been reported only once in 118,482 alleles that were collated by the Exome Aggregation Consortium (exac.broadinstitute.org).

This study extends the description of biallelic mutations in *NTHL1* beyond the single c.268C→T,p.Q90* mutation that was observed previously.[4] Since the cancer phenotype of biallelic *NTHL1* mutations may be very broad, referring to the "*NTHL1* syndrome" could be a useful way of describing this condition, rather than focusing solely on the colorectal phenotype.

Barbara Rivera, Ph.D.
Ester Castellsagué, Ph.D.
Ismaël Bah, M.D.
McGill University
Montreal, QC, Canada
barbara.riverap@gmail.com

and Others

A complete list of authors is available with the full text of this letter at NEJM.org.

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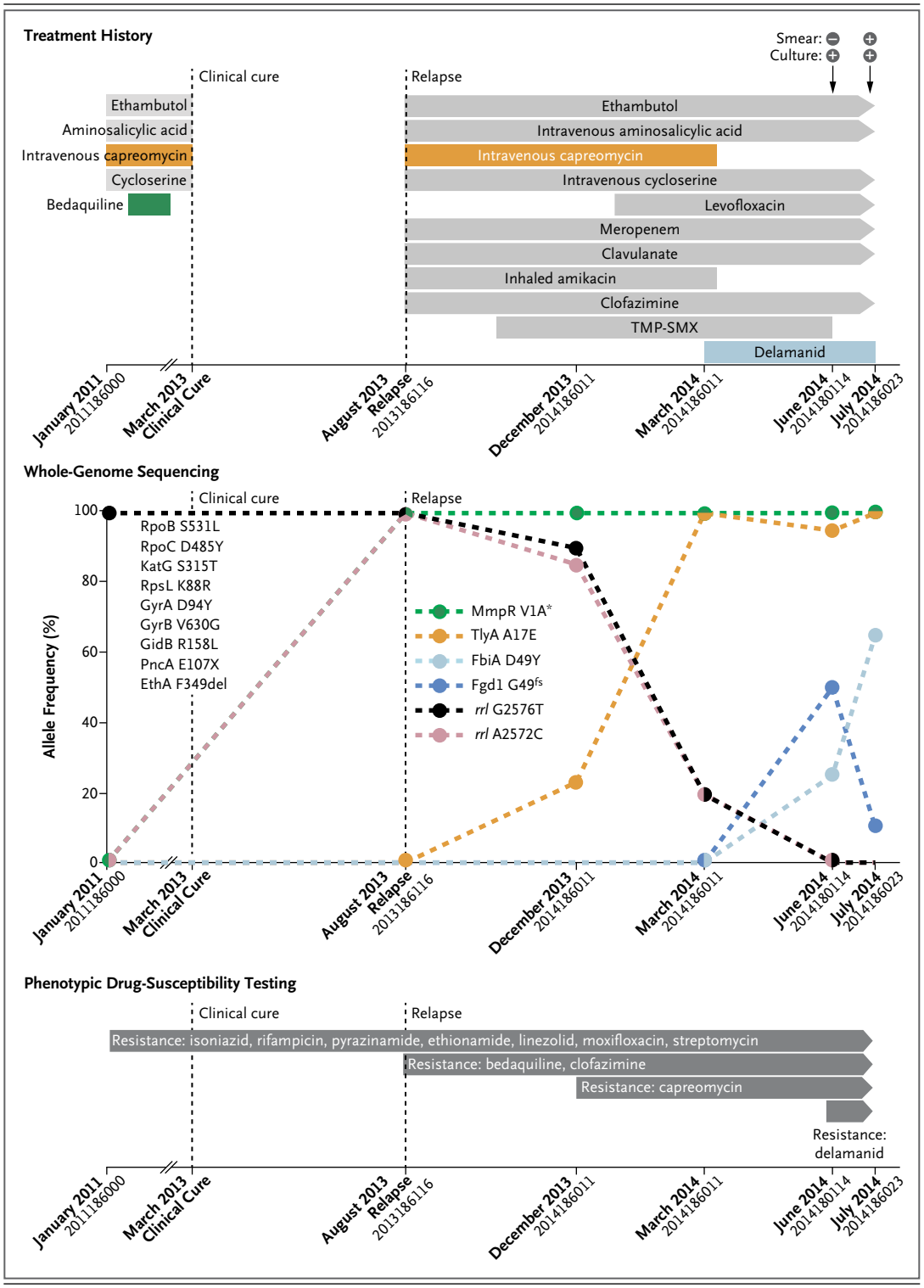
DOI: 10.1056/NEJMc1506878

Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis

TO THE EDITOR: Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are an increasing public health threat.[1] Bedaquiline and delamanid are two drugs that were recently approved by the Food and Drug Administration for treatment of MDR-TB and XDR-TB.[2] Here we describe the stepwise amplification of drug resistance in a patient who had emigrated from Tibet to Switzerland in December 2010 and who presented to a Swiss hospital with preextensively drug-resistant tuberculosis at that time.

Figure 1 (facing page). Clinical Features, Treatment History, Amplification of Drug Resistance, and Phenotypic Drug-Susceptibility Testing in the Patient.

Mutations that confer drug resistance are shown. Resistance to bedaquiline, intravenous capreomycin, and delamanid developed. Percentages on the y axis are based on the number of genome-sequencing reads supporting the corresponding drug resistance–conferring mutation (details are provided in Table S4 in the Supplementary Appendix). Large colored circles indicate measurements, and dashed lines linking these circles indicate the changes in mutation frequency. The loss of the start codon is indicated with an asterisk, and fs indicates frame shift. The mutations listed on the left side of the graph were detected in the first and all subsequent isolates obtained from the patient. All these mutations were seen in 100% of sequencing reads in each isolate. X indicates the stop codon, and del indicates deletion. On the x axis, isolate numbers are listed under the month when the isolate was obtained. TMP-SMX denotes trimethoprim–sulfamethoxazole.



Genome sequencing revealed that the initial *Mycobacterium tuberculosis* isolate harbored nine mutations in genes associated with resistance to seven drugs (Fig. 1, and the Supplementary Appendix, available with the full text of this letter at NEJM.org). The isolate also showed a compensatory mutation in *rpoC*,[3] indicating a "mature" preextensively drug-resistant strain that had evolved

under drug pressure for some time. Given that the patient reported no previous treatment for tuberculosis, he was probably infected with a strain that was already resistant to these drugs.

In September 2011, bedaquiline was added to the regimen, which had consisted of four drugs (ethambutol, aminosalicylic acid, intravenous capreomycin, and cycloserine). The patient was considered to be clinically cured in March 2013, but he had a relapse in August 2013. Genome sequencing of five follow-up isolates revealed a mutation in *mmpR* that was associated with bedaquiline resistance. This mutation persisted even though bedaquiline was discontinued in February 2012; this suggests that it did not cause any clinically significant reduction in the virulence of the infecting bacteria.[4]

Additional resistance to second-line injectable agents (such as capreomycin) also developed. This was reflected in the emergence of mutations in *tlyA* and *rrs*. The latter mutation remained at low frequency and was detected only in drug-containing bacterial cultures (Table S3 in the Supplementary Appendix). All the molecular findings were supported by phenotypic drug-susceptibility testing.

Following this amplification in resistance, delamanid was added to the regimen in March 2014. However, two mutations in *fbiA* and *fgd1* increased in frequency by June 2014, which coincided with the emergence of phenotypic resistance to delamanid. These genes have previously been associated with delamanid resistance. The *fgd1* mutation decreased in frequency thereafter, indicating the presence of multiple delamanid-resistant clones in the patient. In August and September 2014, the patient underwent a lobectomy. After surgery, the sputum specimens obtained from the patient were culture-negative, and the patient received treatment on an ambulatory basis.

This case highlights the development of resistance in the context of inadequate MDR-TB and XDR-TB treatment regimens, despite personalized patient care in a well-resourced health care setting. It serves as a warning for the future rollout of new antituberculosis drugs and emphasizes the need for the use of appropriate companion drugs when bedaquiline and delamanid are administered. Our results add to previous findings showing that the development of drug resistance is a dynamic process involving multiple heterogeneous populations of bacteria within individual patients.[4,5]

Guido V. Bloemberg, Ph.D.
University of Zurich
Zurich, Switzerland

Sebastien Gagneux, Ph.D.
Swiss Tropical and Public Health Institute
Basel, Switzerland
sebastien.gagneux@unibas.ch

Erik C. Böttger, M.D.
University of Zurich
Zurich, Switzerland

and Others

A complete list of authors is available with the full text of this letter at NEJM.org.

Supported by the Swiss Federal Office of Public Health, the University of Zurich, SystemsX.ch, the Novartis Foundation, and grants from the Swiss National Science Foundation (PP00P3_150750), the National Institutes of Health (AI090928), and the European Research Council (309540-EVODRTB).

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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DOI: 10.1056/NEJMc1505196

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